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Bachelor Thesis (15 ECTS)

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Regulation of lipid and protein mobilization in dairy cows during the periparturient period, and the possibility of decreasing plasma NEFA in early lactation

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Abstract

As dairy cows are not able to meet energy requirements for milk production during early lactation, mobilization of fat and protein is initiated. The high mobilization of fat is characterized as high concentrations of NEFA in blood plasma and increases the risk of the metabolic disease ketosis. Protein mobilization is a minor part of the overall mobilization pattern, and the effect and regulation of protein mobilization is therefore not fully accounted for. The possibility that increased protein mobilization could decrease the concentration of ketone bodies was observed in a scientific report from 2012.

To research that possibility, it was necessary to discover what regulates protein mobilization.

The effect of initial body content, endocrine pattern and nutritional supplementation on protein mobilization was investigated. However, as the number of scientific reports covering the subject of protein mobilization is limited, a significant effect of any of these factors was not detected.

The impact of increased protein mobilization on lower BHBA levels was not validated, because in most cases, increased protein mobilization was combined with a decrease in fat mobilization. This situation made it impossible to assign the decrease in BHBA to elevated protein mobilization as the decrease of fat could have caused the same effect.

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Introduction

Through evolution cows and other mammals have developed mechanisms to ensure nutrition to the new-born offspring. These mechanisms act with no regards to tissue maintenance of the mother. Through decades of intense genetic selection to achieve high milk yield, the cow is not able to meet the energy demands required through dietary intake and this results in very low glucose concentration in the blood plasma (Veerkamp et al. 2003).
Low concentration of glucose is causing a high level of mobilization of fat and protein.
The majority of mobilization consist of fat (Komaragiri et al. 1998; Van Knegsel et al. 2007) and is the source to high levels of non-esterified fatty acids (NEFA) in blood plasma. A high concentration of NEFA is associated with increased levels of ketone bodies, which increases the risk of the metabolic disease ketosis (Xia et al. 2012; Mandebvu et al. 2003;). Other diseases such as metritis, lameness and displaced abomasum are found to be connected to high mobilization of fat as well (Suthar et al. 2013).
Several studies are executed in the interest of decreasing the quantity of fat mobilization and thereby the risk of ketosis (Mandebvu et al. 2003; Komaragiri and Erdman, 1997; Komaragiri et al. 1998; Kokkonen et al. 2005; Von Soosten et al. 2012). Regardless of positive results on a few of the trials the overall mobilization of tissue was inevitable.

Van der Drift et al. (2012) investigated the connection between the concentration of plasma β-hydroxybutyrate (BHBA) and mobilization of fat and protein in dairy cows. In the process they observed a correlation between low concentration of BHBA and high mobilization of protein, which is an indication of low ketone production. Van der Drift et al. (2012) believes there's a possibility that increased protein degradation could decrease the risk of ketosis by replacing a larger part of fat mobilization with degradation of protein.

If mobilization is inevitable, then the possibility of shifting the primary mobilization of fat to be of protein instead, could be an advantage in the fight against metabolic diseases caused by high mobilization of fat.

Thesis

Is it possible to regulate the distribution of fat and protein mobilization in lactating dairy cattle during early postpartum and can it have an effect on decreasing plasma NEFA levels?

- How much fat and protein can quantitatively be mobilised during the early postpartum period, and what is the timing of mobilization?
- Does prepartum body condition score have an influence on the distribution between the mobilization of fat and protein during the postpartum period?
- Can an energy supplementation have an effect on the distribution of mobilization during the periparturient period?
- What are the milk production and metabolic consequences of changing the patterns of protein versus fat mobilization?

The above-mentioned hypothesis and questions will be addressed through a literature review.

1. Mobilization of protein and fat during the periparturient period

In order to understand to what extent it is possible to influence the magnitude of fat and protein mobilization in the early lactating cow, it is important to understand the underlying mechanisms regulating the metabolic processes leading to mobilization at tissue level. It is generally agreed upon that the degree of mobilization is linked to the quantity of milk production and thereby nutrient consumption by the mammary gland. But the capacity of nutrition is also important.
The changes in endocrine regulators, induced by nutrient consumption, plays a significant role in governing the adaptation and metabolism in adipose and muscle tissues leading to mobilisation. This mainly include insulin, glucagon, insulin-like growth factor 1(IGF-1),
growth hormone (GH), thyroid hormones (T3 & T4) and leptin. The following will therefore focus on the role of these hormones in regulating the magnitude and composition of the mobilization during the periparturient period.

1. Homeorhetic adaptation to lactation

The dietary intake does not match the high quantity of milk production and therefore creates a negative energy balance characterized by low glucose concentration in blood plasma.
Low glucose levels are the key source to mobilization of both fat and protein.

The reason, why glucose concentration is decreasing to extensive low levels, is that glucose is not distributed equally in the body, but is favoured to certain vital tissues. The distribution of glucose in the body is regulated by glucose transporters, which are influenced by glucose concentration and insulin. The udder along with the central nervous system (CNS), placenta and kidneys
have GLUT1 transporters, which, on very low glucose levels, can utilize almost all of their transport capacity. GLUT 4 transporters on the other hand need higher levels of glucose to influence full transport capacity (Review of Medical Physiology, 20th ed. (ed. W.F. Ganong,s.326)). GLUT1 is ensuring that the udder is one of the first priorities, when it comes to the distribution of glucoses in situations when energy intake is insufficient.
Thus, when lactation has initiated, the udder is utilizing most of the glucose present and leaves the cow in larger NEB, hence a deeper decrease in glucose concentration. (De Vries et al. 1999, Pires et al. 2013).
Glucagon and insulin are both directly but inversely regulated to the concentration of glucose. In

the case of low glucose levels, the concentration of insulin is low and glucagon is high (Aeberhard et al. 2001).
When insulin levels are low, it is affecting the IGF-1 production negatively. Low synthesis of IGF-1 reduces negative feedback on GH synthesis (Peel et al. 1983). This produces high concentrates of GH, together with low IGF-1 in early lactation (Weber et al. 2013; Aeberhard et al. 2001).
As mentioned above, growth hormone levels are high and are very lipolytic (Hansen, 2002) and are main actors in stimulating mobilization of fat in combination with low insulin. Because insulin in high concentrations is inhibiting lipolysis, it is contributing to mobilization of fat when concentration is low (Dimitriadis et al. 2011).
The composition of hormones regulating the mobilization of protein consist of a larger number of components compared to the one of fat.
The explanation is that the sparse stimulation of protein synthesis shifts the balance of protein turnover, because the rate of proteolysis then exceeds the one of synthesis.
The presence of thyroid hormones is stimulating both proteolysis and the synthesis of protein (Brown et al. 1981) and when in NEB, T3/T4 are decreasing and the protein turnover rate is lowered. This mechanism is important because it lowers the energy requirements for maintenance and counteracts the degree of mobilization.
IGF-1 and insulin are the main elements causing the shift in muscle protein turnover, as they both have the effect of stimulating the synthesis of protein in high concentrations (Dimitriadis et al. 2011). Low levels decrease the stimulating effect on protein synthesis and thereby shift the balance of protein turnover, so that the rate of proteolysis exceeds the one of synthesis.

It is clear that the hormonal pattern is favouring the milk production, hence the mobilization, when it comes to energy already digested. But energy intake itself is part of the problem and leptin has an important role in this matter.
Leptin is a hormone released from adipose tissue and thus is an indicator of energy status. When mobilization of fat initiates after parturition it results in decreasing concentration of leptin. Leptin is regulating the appetite via the hypothalamus regulatory centre (Toda et al. 2013).
A general assumption is (Harrison et al. 1995; McNamara et al. 1995) that the dry matter intake (DMI) decreases before parturition, be cause of the cattle pressing against the digestive tract and physically reduces the space for nutrition. After parturition the appetite slowly rises in combination with normal amount of space for nutrition and the lowering of leptin levels. This is stimulating the

appetite and as a result increasing DMI.

1.2. Period of fat mobilization

Looking at the endocrine explanation behind mobilization, leads to the question of when exactly the mobilization of fat and protein occurs during the periparturient period. The scientific trials reviewed to clarify the period of mobilization of fat are presented in table 1, 2 and 3.

The clear indication of mobilization of fat is an increased volume of non-esterified fatty acids (NEFA) in blood plasma, which can be described to degradation of triglycerides (TG) from the adipose tissue (Duffield, 2000)
The mean NEFA concentration before the steep rise at parturition is approximately 0,2 mmol/L (Van der Drift et al. 2012; Chibisa et al. 2008; Weber et al. 2013; Doepel et al. 2002; Kokkonen et al. 2005; Guo et al. 2007) indicating that normal levels of NEFA lies within that range.
The time point of plasma NEFA re-establishing to 0,2 mmol/L hence end of fat mobilization is registered around day 60 postpartum (Van der Drift et al. 2012, Weber et al. 2013 and Kokkonen et al. 2005). Doepel et al. (2002) can partially verify that result by observing 0,3 mmol/L at day 28 indicating that concentration of NEFA is not at initial levels by that time.
Based on the scientific papers reviewed, the time of lipid mobilization occurs from parturition until approximately day 60 postpartum.

1.3. Period of protein mobilization

The scientific reports on protein mobilization during early lactation are limited.
A handful of studies have attempted to measure protein mobilization in the transition period.

Komaragiri et al. (1998) (Table 1 - Appendix) shows loss of body protein in the period of -2 to 5 weeks postpartum by using D2O dilution to estimate body composition at 3 times points respectively -2, 5 and 12 weeks postpartum. The detection of protein mobilization lies, in this study, with the loss of body protein. Even though data of body composition only exist on these time points, the mobilization could potentially have been initiating at -3, -1 , 1 or 2 weeks postpartum. The density and quantity of recording data, results in the method being insufficient when establishing the period of protein mobilization. The same situation occurs in Chibisa et al. (2008) and Komaragiri and Erdman, (1997) which also observes protein mobilization around the time of calving.

Doepel et al. (2002) and Phillips et al. (2003) applied the method of measuring levels of 3-methylhistidine (3-MH). The method is based on the fact that actin and myosin are the contractile proteins of skeletal muscle and during intracellular breakdown of these proteins 3-methylhistidine is released and is therefore an indicator of protein breakdown (Elia et al. 1981; Long et al. 1988). The time of taking blood samples are a week apart and thereby shows a better picture of when protein mobilization occurs.
Looking at the results given by Doepel et al. (2002) the levels of 3-MH could indicate that protein mobilization starts within 2 weeks before calving, as an increase in concentration takes place, and an indication of stabilization around week 4 postpartum. This unfortunately in combination with the end of trial. Further recordings might have verified the termination of protein degradation. However, the same pattern of 3-MH concentrations are observed in Phillips et al. (2003) and the hypothesis that 3-MH levels stabilizes around week 4 is confirmed in this trial, which have data that continues until week 17 postpartum. Van der Drift et al. (2012) applied the 3-MH method as well and confirms the end of mobilization at week 4 postpartum. But the initiation of protein degradation is not validated to be as early as 2 weeks before calving but at approximately 1 week before.

Based on current literature reviewed above, the period of protein mobilization probably takes place approximately 1-2 weeks before calving and last until 4 weeks postpartum.

1.4 Quantitative amounts of mobilization of lipids and proteins

If stating that protein mobilization occurs within the period of week -2 until week 4 postpartum, the importance of having data in that period to estimate the quantity of protein mobilization is great. Von Soosten et al. (2012) *(Table 1 - Appendix)* has data recorded from 1 day in lactation to 105 days in lactation and do therefore not include the whole period of protein mobilization. On that ground, the estimations have the possibility of being inaccurate and will not be considered regarding the quantity of protein mobilization.

Two scientific reports (Komaragiri and Erdman, 1997 and Komaragiri et al. 1998) utilized the exact same method to measure body composition, with approximately the same amount of animals (n = 22 and 26) and got estimations of the quantity of protein mobilization far from each other. Respectively 4,8 kg in cows in control group (komaragiri et al. 1998) and 20,9 kg for all cows (Komaragiri and Erdman, 1997). The only difference in the two trials seems to lie within initial body protein store. Respectively 71 kg protein and mean of 94,7 kg initial body protein. However the degree of which initial protein stores can verify the difference in protein mobilization is not clear. And because diets in both trials are too similar to create such difference in protein mobilization, it is not possible to explain the difference any further, other than potential problems in relation to measuring techniques or calculations.
The initial body protein content might have an effect as a loss of protein of 13,6 kg with high initial body protein at 112,6 kg is observed in cows not treated with propylene glycol(control) in Chibisa et al. (2008) *(Table 1 - Appendix).*
A parameter to create a form of common basis could be % crude protein of DM, as it is dominant factor in some of the trials and it is possible to find information regarding this parameter on most of the other reports.
Phillips et al. (2003) divided the cows into two groups assigned either diet containing 11 % crude protein (CP) or 14 % CP. The group of cows fed closest to other studies according to % protein, in Komaragiri and Erdman, (1997) were fed 16 % CP and the comparison must therefore be with the cows fed 14 % CP (Phillips et al. 2003) which showed a protein mobilization of 14 kg.

With Chibisa et al. (2008) which fed 17,3 % CP in control group and percentage of crude protein was not available in Komaragiri et al. (1998) a mean of the result selected above in respectively Komaragiri and Erdman., (1997), Chibisa et al. (2008) and Phillips et al. (2003) gives the best estimate of the quantitative aspect of protein mobilization. Time of recording within these three trials are not including any periods of potential protein accretion as the longissimus muscle does increase in size before week 8 postpartum (Van der Drift et al. 2012). The mean is given as approximately 16 kg of protein and is calculated without the regard of initial protein content possible effect on protein mobilization.

In the respect of quantifying the mobilization of fat, results from Von Soosten et al. (2012) can be

of use as mobilization, discussed in earlier chapter, initiates at parturition and observed to 24,1 kg of fat by day 42. Results extracted from data recorded at day 42 might be underestimated as mobilization of fat in general decreases around day 60 postpartum as discussed in earlier chapter. The reason for selection of results from trials discussed forward, are similar to the ones applied with the quantification of protein mobilization.
Observations from Komaragiri et al. (1998) at 65,9 kg fat and Komaragiri and Erdman, (1997) at 59 kg fat might be slightly underestimated, explained by estimations are calculated from data recorded at d. 114 where accretion of fat might have occurred if day 60 is the end of fat mobilization. An underestimation of the observation on fat loss at 21 kg in Chibisa et al. (2008) is present due to the fact that recording of data ceased at day 38. Ending trial and recording at day 60 (Phillips et al. 2003) presents a fat mobilization at 56 kg. This is in agreement of 21 and 24,1 kg of fat being an underestimation, however not of 59 and 65,9 kg being underestimated. The quantity of lipid mobilization probably lies within the range of 30-50 kg of fat.

2. The effect of initial body content on mobilization of body tissues

When reviewing the studies in the previous chapter, an opportunity that initial body tissue content could be affecting mobilization of fat and protein is present. Thereby leading to further assessment on this topic in current chapter.

Pires et al. (2013) are testing body condition score (BCS) on mobilizations of body tissues by analysing plasma NEFA, 3-MH and 3-MH/creatinine ratio. 28 cows were allocated into 3 groups, high-, medium- and low-BSC, according to their initial BCS value. Postpartum NEFA levels were significantly higher in high-BCS compared to both medium- and low-BCS. The 3-MH/creatinine ratio was significantly higher in low-BCS indicating higher protein mobilization. The effect was not validated by 3-MH levels alone despite numerical greater levels of 3-MH in low-BCS than observed in other groups.
The procedures in estimating BCS are done by overlooking parameters such as size of the cow (Bazin et al. 1984) and thereby add insecurity upon the distinction between fat and protein contents on the cow. To investigate the influence of protein and fat content separately, other measurements are thereby necessary to utilize.

The initial body fat content is positively correlated to the amount of fat mobilized (Komaragiri et al. 1998; Chibisa et al. 2008; Kokkonen et al. 2005; Komaragiri and Erdman, 1997 and Phillips et al. 2003). The results of these studies all indicate, by tendencies, that higher initial body fat content increased the amount of fat mobilization. By the exception of Komaragiri et al. (1998), the same scientific reports showed tendencies of cows with higher initial protein content mobilized more protein. It is to be mentioned that tendencies in most articles reviewed are extremely insignificant however it does not exclude the possibility that initial body content has an effect on the amount of lipid and protein mobilization to some extend.

3. Effect of nutritional supplementation on mobilization

Nutritional supplementation has been widely tested to find a source to decrease high mobilization of fat. As the essential of this paper includes both fat and protein mobilization, the number of studies are restricted by the fact that they need to cover both metabolisms.

3.1 The effect of dietary protein on mobilization

Three scientific papers (Komaragiri and Erdman, 1997; Phillips et al. 2003; Doepel et al. 2002) all investigated the effect of dietary protein on body tissue mobilization during the periparturient period, by treatment of two different amounts of CP added to diet either prepartum or during the complete period. A short review is presented in table 2.
The outcome from the reports presents some differences, but methods utilized to achieve these have basic differences as well. Komaragiri and Erdman, (1997) used a D2O dilution method to estimate body composition and finds no effect of increasing dietary crude protein on the mobilization of protein. However, they did find a larger effect on the mobilization of fat with an increase of almost 15 kg. In earlier discussion, the question was raised regarding a possible error during estimation of body composition in either Komaragiri and Erdman, (1997) or Komaragiri et al.(1998). Utilizing body composition, derived from dilution method, to estimate the effect of dietary protein on mobilization is also applied in another trial (Phillips et al. 2003) which observes an increasing effect on protein degradation when fed additional crude protein. In the same study this statement is invalidated by a contradictory result from levels of 3-MH and 3-MH/creatinine ratio indicating higher CP lowers protein mobilization.

The decreasing effect of dietary protein on protein mobilization is confirmed by a tendency of increased CP lowering concentrations of 3-MH hence protein degradation (Doepel et al. 2002) There seems to be a larger agreement upon the effect of crude protein in diet, when applying the method of 3-MH in comparison to variations of dilution methods.
Regarding the effect of dietary protein on the mobilization of fat, results from Doepel et al. (2002) were inconclusive and Phillips et al. (2003) showed no effects. Komaragiri and Erdman, (1997) was the the only one to record a response of change in the amount of crude protein and the report separates by feeding additional protein throughout the trial, while the 2 other studies end feeding at parturition. The time of experimental feeding period makes Komaragiri and Erdman, (1997) incomparable to the other studies reviewed in this chapter regarding the effect of dietary protein.

Based on the review of the studies mentioned above, additional dietary protein may have an decreasing effect on protein mobilization when added in the prepartum period. The effect on the mobilization of fat is unclear.

3.2 The effect of dietary glucose on mobilization

The general hypothesis of adding a variation of glucose is to decrease plasma NEFA concentration (Miyoshi et al. 2001; Pickett et al 2003; Wang et al. 2009) The impact of adding glucose on protein mobilization is not investigated to the same extent.

Results observed in two trials (Chibisa et al. 2008 and Kokkonen et al. 2005) were not significant and in the same time contrary. Chibisa et al. (2008) added propylene glycol (PG), which is a precursor to the metabolism of propionate in ruminants. Propionate is a short-chain fatty acid (SCFA) which is the primary source to glucose in ruminants, as it is a precursor in gluconeogenesis (Drackley et al. 1999). The object of this study has thereby been to add glucose via propylene glycol in diet. Using plasma NEFA levels and urea dilution technique to estimate body composition there was no significant effect on either fat or protein mobilization, although cows fed PG had an average decrease in fat loss from 21 kg to 18,6 kg and an increase in protein degradation from 13,6 kg to 15 kg. Chibisa et al. (2008) is confirming the general hypothesis on the decrease of NEFA and the result regarding protein mobilization might therefore be a possible effect. (Kokkonen et al. 2005) tested to see if a glucogenic supplementation (GS) could lead to any changes regarding mobilization of body tissues using scanning of longissimus muscle and subcutaneous fat depth to measure the effect.

The results on the effect of GS on protein mobilization were inconclusive but a very small increase was observed in fat mobilization.

4. The effect of increased fat and protein mobilization on milk performance

When looking into the possibility of regulating the distribution between lipid and protein mobilization it is important to recognize the consequences of this action. Thereby leading to the question on the impact of the magnitude of protein and fat mobilization, on the milk performance and on other metabolic consequences.

Mobilization of fat means an elevated concentration of NEFA in plasma. Higher levels of NEFA are often seen to result in higher milk fat percentage. Komaragiri and Erdman, (1997), Doepel et al. (2002) have data indicating an increase of NEFA combined with a tendency to have increased milk fat percentage in one group of cows compared to the group with lower levels of NEFA *(table 3 -Appendix).* Theoretically, these tendencies make sense as NEFA absorbed by mammary glands are positively correlated to the availability of plasma NEFA. Thus, elevated NEFA levels, as an indicator of fat mobilization, would increase milk fat percentage. Contrary, Komaragiri et al. (1998) ran a trial with added fat to diet and observed an increase in protein mobilization and decrease of fat mobilization. The effect of milk fat % still increased, only by 0,04 %, and a decrease in milk protein percentage was also spotted. The milk fat % was expected to decrease in combination with low fat mobilization and the small increase might be due to the added fat. The decrease in milk protein is not clear, but the observation of increased milk yield could explain the decrease in percent if the rate of protein synthesis was not elevated in combination with milk yield. However, milk protein synthesis has a genetic maximum and the possibility of increasing milk protein percentage is therefore not endless.

So in a case with very high protein mobilization the result would not be a matching high protein in milk but an increased energy balance as the available amino acids can be used in hepatic gluconeogenesis

5. Metabolic consequences

The metabolic consequence of high mobilization of fat in lactating dairy cows is often fatty liver, ketosis, metritis, lameness and displaced abomasum (Suthar et al. 2013). The effect of high mobilization is greatly validated by several scientific reports and will not be assessed further. On the other hand, the metabolic consequences of increased protein mobilization in dairy cows have not been covered in the same extent. An analysis of 2 reports having recorded a change in protein mobilization and traits that indicate metabolic status will be assessed beneath.

Chibisa et al. (2008) observed an increase in protein mobilization in a group of cows fed a glycogenic supplement (GS) compared with group not fed GS. BHBA levels were registered for both groups however the groups took turn in having the lowest BHBA concentrations during lactation and the effect of increased protein mobilization on production of ketone bodies is thereby inconclusive. On the contrary, another study (Doepel et al. 2002) had results of levels of BHBA that were not inconclusive and did in fact show that increased 3-MH hence increasing protein mobilization tended to have lower levels of BHBA. However, the NEFA levels in this trial were inclusive which made it impossible to know if the same group was mobilizing less fat which could have been the cause of the decrease of BHBA.

6. Discussion

Literature indicated that at day 60 postpartum the NEFA concentration tend to decrease back to normal levels. The time of entering positive energy balance seems to occur around day 60 postpartum (Kokkonen et al. 2005; Weber et al. 2013; De Vries et al. 1999)

The negative energy balance thereby shows when the cow is mobilizing fat as the period of negative energy balance matches the one of mobilization and confirming the period and cause of mobilization of lipids.

Validating the results extracted from the period of protein mobilization was not possible, when using endocrine parameters based on literature assessed.

When looking at the endocrine regulation of protein mobilization, low concentrations of IGF-1 and insulin are the key elements. However, following the pattern of insulin, IGF-1 and protein mobilization the match is not complete. As protein mobilization tend to cease around week 4 into lactation the levels of insulin and IGF-1 does not seem to elevate around the same time point, but continues to stay low (Pires et al. 2013: Doepel et al 2002). However, Kokkonen et al. (2005) and Van knegsel et al. (2007) observed, around week 4, that insulin levels were increased to the similar level as when protein mobilization initiated, around week 1-2 prepartum. The fact that there is not a clear picture might be due to incorrection in estimating time of protein mobilization or the hormonal regulation. But as the scientific literature existing on the time of protein mobilization in lactating dairy cows is limited and thereby increase insecurities on these estimation, thus questioning the knowledge of basic protein metabolism is not wise.

In the matter of explaining the time of protein mobilization other theories have been presented. Initiation of protein mobilization might be due to other factors than low energy status, such as protein deficiency as the need for more protein during the last weeks is greater caused by increased fetal growth.

The statement that a greater need for protein increases protein mobilization is confirmed by the overall tendency of adding protein to diet decreased protein mobilization. On the other hand it is invalidated by higher initial protein content which seemed to increase protein mobilization.

A problematic situation occurred several times, caused by the use of the same reports to test different effects on mobilization. In Komaragiri et al. (1998) an increased protein mobilization was observed in the group of cows being fed additional fat compared to the control group. The group fed added fat was at the same time the group with higher initial protein content. It was in this case not possible to know which of the factors caused the increase in protein mobilization.
Many of the tendencies extracted from the scientific reports is within the assumption of other elements not being the cause of change. Although problematic, these precautions were necessary if any assumptions, according to regulation of protein mobilization, were to be made.

It is not clear if initial body fat content is increasing NEFA levels because of more available fat or indirectly by having higher leptin levels that decrease appetite resulting in lower DMI and thereby higher negative energy balance.
In continuance, it is known that hormones such as leptin self regulates back to normal concentrations when having been elevated for a longer period of time. If this situation were present in some of these trials, the possibility of high leptin causing higher fat mobilization could be ruled out, and validate the effect of initial fat content prepartum on fat mobilization in lactation. The measuring of leptin levels in combination with initial body fat content could have clarified that, but was not applied in reports where the extraction of initial body fat content was taken from with the exception of Kokkonen et al. (1998). The results from that study can not rule out the effect of leptin on higher fat mobilization, as the levels of leptin were inconclusive in the group (G1) where higher initial fat content indicated increase in fat mobilization *(table 2 - Appendix).*

Primiparous cows are still growing and energy requirements is higher than for maintenance, and plus the udder is not fully grown hence milk production capacity is smaller than compared to multiparous cows. The influence on the mobilization of fat is explained by the fact that energy requirement for lactation is minor than in full grown cows, with fully developed udder and thereby result in a smaller negative energy balance (De Vries et al. 1999). Taken this into consideration, some of the conclusions made during this paper might have looked different as a report, referred to repeatedly, included both primiparous and multiparous without distinguishing between them (Chibisa et al. 2008). In addition, when comparing a result from a report using primiparous cows to one using multiparous, the importance of parity might have change the outcome of the conclusions.

The impact of increased protein mobilization on lower BHBA levels can not be validated, because in most cases, increased protein mobilization was combined with a decrease in fat mobilization. It is therefore impossible to assign the decrease in BHBA to elevated protein mobilization as the decrease of fat could have caused the same effect.

7. Conclusion

The time, quantity and regulation of protein mobilization during the periparturient period are subjects in lack of research, therefore theories and estimation often stand by themselves and are difficult to validate. It is important to investigate the influence and regulation of protein mobilization further, as a clear answer on whether increasing protein mobilization might decrease the NEFA concentration and risk of developing ketosis did not appear from current available scientific reports as several other factors could have been of influence in cases where this effect was detected.

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Table 1. Short review of scientific reports

	Komaragiri et al. 1998	Chibisa et al. 2008	Von Soosten et al. 2012

Parameters to record fat and protein mobilization	D2O-dilution method to estimates body composition. Which uses general means of ratio of empty body protein, empty body water and empty body ash.	Urea dilution technique to measure body composition. Blood samples to define levels of BHBA, NEFA.	Comparative slaughter technique at different time points.
Treatment	1 group fed control diet and another group fed control diet plus 3% added fat (on dry matter basis)	1 grp on control diet and 1 grp on control diet plus 600 mL/day of propylene glycol (PG)	1 grp on control diet (CON) and 1 grp on control diet plus conjugated linoleic acid (CLA).
Time of treatment	2 weeks prepartum to 12 weeks postpartum	Day -7 to 45 according to calving.	Day 1 to day 105 postpartum.
Number and time of samples	3 samples/cow during study at -2, 5 and week 12 postpartum.	3 samples/cow. All measurement were taken at day -14 +/- 5, 15 and 38 relative to calving.	Slaughter of 5 cows from CON on day 1, slaughter of 5 CON and 5 CLA on day 42 and last slaughter of 5 CON and 5 CLA on day 105.
Initial BW/Breed	Grp Control: initial protein at 71 kg and 159,2 kg of fat. Grp fat: initial protein at 82,4 kg and 126,2 kg fat /Holstein	Grp Control: initial protein at 112,6 kg and 125,9 kg of fat. Grp PG: initial protein at 110,1 kg and 121 kg fat / -	• /Holstein
Parity	Multiparous	7 primiparous and 9 multiparous	Primiparous
Animals in study	22 cows - assigned two groups.	16 cows paired according to calving date and each pair were randomly assigned the control diet or a PG diet.	25 cows
Results of study according to the ***quantitative mobilization*** *of protein and fat.*	All results was insignificant. Loss of body protein: Control grp: 4,8 kg Fat grp: 19,1 kg	Protein mobilization: Control grp: 13,6 kg PG grp: 15 kg	Result from day 1 til d 42: CON lost 24,1 kg fat and gained 2,8 kg protein.

	Loss of body fat: Control grp: 65,9 kg Fat grp: 37 kg Estimates for week -2 til 12 postpartum.	Fat mobilization: Control grp: 21 kg PG grp: 18,6 kg	CLA lost 14,3 kg fat and gained 3,6 kg protein.
*Results of the study according to the **effect of treatment** on protein and fat mobilization*	The results were not significant. Increase of protein loss from 4,8 kg to 19,1 kg in fat grp. And a decrease of fat loss from 65,9 kg to 37 kg in fat grp.	Results were far from significant. PG decrease fat mobilization from 21 kg to 18,6 kg. PG increased protein mobilization from 13,6 kg to 15 kg.	-
*Results of the study according to **initial body score having an effect on protein and fat mobilization***	Initial body protein: Control 71 kg protein Fat grp 82,4 kg protein Initial body fat: Control grp. 159 kg fat Fat grp. 126,2 kg fat Highest initial body fat mobilized more fat. Highest initial body protein lost more protein. NOT significant.	Initial body fat were higher in control by 4,9 kg which lost more fat. Initial body protein were higher in control which lost the least amount of protein.	-
*Results of the study according to **effect of treatment on milk yield and milk composition***	None of the changes were significant: Fat grp showed an increase in milk yield from 39,9 kg/d to 42,6 kg/d. And fat increased % of milk fat with 0,04% and decreased % of protein in milk with 0,11 %.	Results are highly insignificant: PG produced less milk than control and had a higher % of fat in milk. And had lower % of lactose and protein in milk.	-

Table 2. Short review of scientific reports

	Van der Drift et al. 2012	Weber et al. 2013	Kokkonen et al. 2005

Parameters to record fat and protein mobilization	Fat: plasma NEFA and backfat thickness. Protein: plasma 3-MH and length of longissimus muscle	Protein: NONE! Fat: Plasma NEFA	Ultrasonographic scanning of longissimus muscle volume and subcutaneous fat depths. Plus NEFA and creatitinine
Treatment	No treatment - just observation of animals	No treatment, but divided into groups by liver fat content - low, medium and high after calving	test (T) group were with cows with the highest initial body fat. control (C) group with the lowest initial body fat. Both C and T were either on (G1) or of glucogenic (G0) supplementation diet from day -14 until day 56 postpartum.
Time of treatment	Observation from -4 until week 8 postpartum	Day - 56 to day 63 according to parturition	day -14 until day 56 postpartum.
Number and time of samples	At -4, -3, -2, -1, 1, 2, 3, 4, 5, 6, 7 and at week 8 postpartum.	Blood samples taken at d 56, 28, 15, and 5 before calving and at d 1 , and once weekly up to d 63 postpartum.	Muscle volume recorded 5 times: week -8, -1, 0, 1 and 4 postpartum. Adipose tissue samples: at -1, 0, 1 and 4 weeks postpartum.
Initial BW/Breed	/Holstein-Friesian and Holstein-Friesian crossbreed.	/Holstein	/Ayrshire
Parity	Primi- and multiparous	Third lactation cows	Multiparous
Animals in study	34	27	24 - cows were divided into blocks of 4. Two cows with the highest body condition score within

			each block were then allocated to a test (T) group and the other 2 cows to a control (C) group.
*Results of study according to the **period of mobilization of fat.***	NEFA levels drops at first sample taken at 1 week postpartum and stays above initial concentration throughout trial at week 8 postpartum. Backfat thickness decreases at week 1 and keeps decreasing throughout the trial.	The mean of NEFA increased at parturition and fell to almost initial level at end of trial at day 63	-
*Results of study according to the **period of mobilization of protein.***	3-MH concentration increases at -1 week postpartum and decreases to stabilizing levels at week 4 postpartum.	-	-
*Results of study according to the **quantitative mobilization** of protein and fat.*	-	-	-
*Results of the study according to the **effect of glucose on protein and fat mobilization***	-	-	None of the results were significant. (G1) showed no tendencies of affecting protein mobilization. (G1) tended to increase fat mobilization by a mean of 37 % *
*Results of the study according to **initial body score having an effect on protein and fat mobilization***	-	-	(T) tended to increase loss of body fat and protein mobilization
*Results of the study according to **initial body score having an effect***	-	-	NO tendencies of (T) having effect on the composition of milk.

on milk yield and composition			Milk yield increase by a mean of 0,9 kg/day in (T), not significant
Results of the study according to ***effect of glucose on milk yield and composition***	-	-	(G1) tended increase milk yield with 1,8 kg/day. (G1) had no effect on the composition of milk.

*Calculations are to find in appendix. (Fat: increase from a loss of 1,1 mm to 1,3mm ~ increase of 27% . and from loss from 1,9mm to 2,8mm ~ increase in loss of 47 % - the mean of increase in loss of body fat depth are 37%)

Table 3. Short review of scientific reports

	Komaragiri and Erdman, 1997	**Phillips et al. 2003**	**Doepel et al. 2002**
Parameters to record fat and protein mobilization/ Body composition	D2O-dilution method to estimate body composition. Which uses general means of ratio of empty body protein, empty body water and empty body ash.	Fat cell size and body weight used to calculate body protein and body fat via equations. Levels of 3-MH and 3-MH/creatinine ratio.	Blood samples where Analyzed for levels of 3-MH, NEFA(non-esterified fatty acid), BHBA(ketone body), 3-MH/creatinine ratio
Treatment	2 dietary treatments with different crude protein levels: 16 % or 19 % CP.	2 CP levels fed: 11 or 14% CP and with or without a methionine hydroxy analog (AT88) - giving 4 different groups. Low protein w. AT88, Low Protein no AT88, High protein w. AT88 and High protein no AT88.	2 energy levels fed: 1,65 Mcal/kg(HE) of net energy for lactation (NEL) and 1,30 Mcal/kg(LE) of NEL 2 protein levels fed: 17 %(HP) and 12,5 %(LP) crude protein (CP) - giving 4 different treatment: HEHP, HELP, LEHP and LELP.
Time of treatment	2 weeks prepartum to 12 weeks postpartum	21 days prepartum to parturition - all fed diet with 17 % CP with or without AT88 for the 120 days postpartum.	21 days prepartum to day of parturition - fed same diet postpartum.

Number and time of samples	3 samples/cow during study at -2, 5 and week 12 postpartum.	Blood samples at: -7, 0, 7, 14, 28, 56, 84 and day 112 postpartum. Fat cell size samples at: -14, 60 and day 120 postpartum.	Blood samples at: -21, -14, -7, 0, 7, 14, 21 and day 28 postpartum.
Initial BW/Breed	Initial mean weight of all cows 760 kg. Grp 16CP: initial protein at 94,5 kg and 142,6 kg of fat. Grp 19CP: initial protein at 94,9 kg and 185,5 kg fat / Holstein	Measured but not shown/ Holstein	• /Holstein
Parity	Multiparous	Multiparous	Multiparous
Animals in study	26 randomly split into 2 groups of 10	42 - blocked by parity ('2' , '3 or higher') and then allocated to 4 treatment groups of 11, 10, 9 and 12.	26 - blocked by parity, milk production, expected calving date and then randomly allocated into 4 treatment groups.
Result of study according to ***estimation of the period of protein mobilization****.*	Protein mobilization was observed in the period of week -2 and 5 postpartum	Levels of 3-MH and 3-MH/creatinine ratio indicates protein mobilization -2 to week 4 postpartum	Levels of 3-MH indicates protein mobilization from week -2 to 4 postpartum.
Results of study according to the ***quantitative mobilization*** *of protein and fat.*	Result from week -2 to 5 postpartum: Grp 16CP lost: 20,9 kg protein and 45,3 kg fat. Grp 19CP lost: 20,9 kg protein and 60,5 kg fat. Result from week -2 to 12 postpartum: 16CP lost 59 kg fat 19CP lost 82,5 kg fat	Result from d -14 to 60 postpartum: Low CP and high CP lost the same amount of fat of approximately 56 kg. Low 11%CP lost 11 kg protein and high 14% CP 14 kg protein.	-

	Change in protein loss were the same as by week 5.		
Results of study according to the ***effect of dietary protein on mobilization*** *of protein and fat.*	No effect of CP on the mobilization of protein. An increased amount of fat was mobilized from treatment with 16CP to 19CP between week -2 to 5. A loss of fat of 45,3 kg to 60,5 kg respectively. Although the difference was not significant.	Result from -14 to 60 days postpartum: No difference in fat mobilization due to difference in % CP. The loss of body protein ranges from 11-14 kg, with high CP losing the greatest amount. The statement of high CP losing greater amount of protein is not confirmed as a tendency for high CP having the lowest levels of 3-MH and the ratio 3-MH/creatinine.	Fed high CP tended to decrease 3-MH postpartum. A significant increase of BHBA postpartum when fed high CP. LEHP showed the greatest level of NEFA postpartum. Values on NEFA concentrations were inconclusive according to dietary protein having any effect on fat mobilization.
Result of study according to the ***effect of protein and fat mobilization on milk yield and composition of milk***	19CP tended to mobilize more fat and at the same time tended to increase milk fat % As there was no change in protein mobilization it is not clear to see if it had an effect.	Fat mobilization were the same and potential effect is there detectable. The state on whether a difference in protein mobilization is observed is inconclusive as 3-MH levels and measurements on body composition are contradictory.	An increase in overall dietary energy(HE) tended to decrease NEFA around the time of calving and tended in the same period to decrease milk fat %
Results of study according to the ***effect of dietary energy on mobilization*** *of protein and fat.*	-	-	An increase in overall dietary energy(HE) tended to decrease NEFA around the time of calving. A tendency of general increased energy balance for (HE)
Results of the study according to ***initial body score having an effect on protein***	Initial body protein for 16CP: 94,5 kg and 19CP : 94,9 kg with approximately the same protein	With 2 different amounts of initial body fat the cows lost the same amount of fat. No correlation between initial body fat and fat mobilization observed.	-

and fat mobilization	mobilization at 20,9 kg. Initial body fat for 16CP: 142,6 kg and 19CP: 185,5 kg with a mobilization of respectively 45,3 kg and 60,5 kg.	Cows with the highest initial body protein tended to mobilize more protein.	
Results of the study according to ***effect of treatment on milk yield and milk composition***	19CP increased milk yield from 39,4 to 42,4 kg/d. Small increase in fat % in milk with 19CP by 0,06 %. Small increase in protein % in milk with 19CP by 0,19 %. Plus an increase in lactose % in milk by 0,05 %.	None of these results are significant: Higher CP tended to increase milk yield from 40,6 kg/d to 42,3 kg/d. High CP tended to decrease the % of protein, lactose and fat in milk.	(HE) tended to increase milk yield with: Period week 1-3; 3,1 kg/d with (HP) and 2 kg/d with (LP) Period week 4-6; 4 kg/d with (HP) and 0,7 kg/d with (LP). (HE) tended to lower % of milk fat in week 1-3, but not in week 4-6. Protein had no tendency of affecting % of milk fat at all. No tendencies of either (HE) or (HP) effecting % of protein or lactose in milk.